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THE DIAGNOSIS OF RABIES IN ANIMALS.

A STATISTICAL STUDY OF THE RECORDS OF THE HYGIENIC LABORATORY FOR THE PERIOD 1909 TO 1919.

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This study, from the records of the Hygienic Laboratory, was undertaken to determine the reliability of the microscopical examination for the presence of Negri bodies as a means of diagnosing rabies. From February, 1909, to April 30, 1919, 1,127 specimens from suspected animals of various species have been submitted. While the examinations have been made by a number of officers of the Public Health Service, the general technique followed has been practically constant throughout.

Technique of tests.

A description of the technique employed will serve to make clear the different types of examination. Three types of tests have been used which may be designated as the smear, the section, and the inoculation tests. Some brains were subjected to all three tests, but after a time this was discontinued as it was apparent that when Negri bodies were found by microscopic examination the inoculation test was almost invariably positive. Consequently the inoculation test was used only in case the microscopic examination failed to show Negri bodies, or in cases in which the bodies found were of doubtful character.

The *smear test*, advocated by Williams and Lowden, is performed as follows: A thin section of the hippocampus major (Ammon's horn) of the brain of the suspected animal is removed and placed on a clean glass slide. It is then crushed between two slides and the slides drawn apart making a uniform smear of the brain tissue upon both slides. The smear is flooded with methyl alcohol (ethyl alcohol may be used) for one minute. This is poured off and the smear is stained with fuchsin-methylene blue, the preparation being steamed slightly for about one minute. The stain used by the Hygienic Laboratory is prepared fresh each day as follows: To about 50 c. c. of distilled water add 3 drops of saturated alcoholic solution of basic fuchsin and about 5 drops of saturated aqueous solution of methylene blue. Mix well and use at once. If the smear is too red, or too blue, the stain may be modified by adding 1 or 2 more drops of the stain desired and repeating the staining process on another smear.

After the smear has dried it is examined as follows: With the low-power lens a field showing large pyramidal cells of the hippocampus major is located. The field is then covered with a drop of oil and examined with the immersion lens. The large cells should be stained blue; the Negri bodies, if present, are stained red and found lying within, or near, the cells. Closer examination usually reveals one or more small blue dots within the Negri bodies, though at times these are difficult to demonstrate.

To make a positive diagnosis Negri bodies should be found *within* the cells, though the method of preparation often results in bodies being found outside the cells. Owing to the resemblance of red blood cells to the extracellular bodies, the finding of intracellular bodies is the criterion of a positive diagnosis.

If the smear gives a positive result, no further test is made. If negative, a section of the hippocampus major may be taken and placed in fixing solution for the preparation of sections. If Negri bodies are not demonstrated by the smear method, a portion of the brain (hippocampus, medulla, or other part) is placed in glycerin for about three days, to be used for the inoculation of animals.

Before recording a negative finding by the smear method, several smears should be stained and examined, and they should be made from the hippocampus major of both cerebral hemispheres. In a few instances Negri bodies have been found easily in one hippocampus when smears from the opposite side have failed to reveal their presence.

The *section method* is a little more accurate than the smear method but the longer time required and the greater amount of work necessary in that method make the smear method the more practicable. In a few cases the section test has been the only one possible, as the brains were submitted after having been placed in formaldehyde or other fixing solution.

For demonstrating Negri bodies in sections we have found that Zenker's solution as the fixing fluid and the eosin-methylene blue stain give the best results. We have used paraffin for embedding and cutting sections. Using this technique the cells of the hippocampus are stained blue and the Negri bodies red with the blue inner bodies usually showing. All the bodies should be within the cells as the method of preparation should not break up the cells.

The *animal inoculation method* is used in all cases which give a negative microscopic finding. A piece of the brain tissue of the suspected animal is placed in glycerin for from three to five days. It has been found that this treatment serves to lessen the danger of abscess or other lesion which may result from contaminating organisms. Animals inoculated with a brain emulsion made without this preliminary period in glycerin frequently die in the first 3 days, a period far too short to be of any value in the diagnosis of rabies.

After the suspected brain tissue has been in glycerin for from 3 to 5 days, a thick emulsion of the tissue is made by grinding it in a sterilized mortar and adding sterilized salt solution. A small amount, about 0.2 c. c., of this emulsion is then injected with a sterile syringe subdurally, or intracerebrally, into guinea pigs or rabbits. The animal is etherized, the skin across the parieto-occipital region is incised, and a small hole is punched in the cranium with the center pin of a trephine. The needle is passed through this hole and the desired amount injected. After the operation a collodion dressing may be applied to the skin incision if desired. At least 2 animals are inoculated from each suspected case. The animals are then observed for symptoms of rabies, and if they die after a period of 5 days an examination of their brain for Negri bodies is made. Animals should be kept under observation for at least 6 months; though most of those developing rabies will develop symptoms within 2 months.

Origin and Distribution of Specimens.

While the specimens examined came from different parts of the United States, a large majority came from the District of Columbia, Maryland, and Virginia. A greater number of specimens from the District of Columbia were examined by the Bureau of Animal Industry, Department of Agriculture, and many specimens from Maryland were examined by the health department of that State. While the figures given in the following table do not indicate the true prevalence of rabies in any State, the geographic origin of specimens by States is shown.

TABLE I.—*The geographic origin of specimens, by States.*

State.	Total.	Positive.	Negative.	Not examined.
Arizona	2		2	
District of Columbia	24	6	16	2
Florida	3	1	1	1
Kentucky	7	2	3	2
Maryland	42	26	15	1
Mississippi	1		1	
Nebraska	1		1	
North Carolina	2	1	1	
New Mexico	4		4	
New York	2	1	1	
Oregon	1	1		
Pennsylvania	1	1		
South Dakota	1		1	
Tennessee	4	1		3
Virginia	1,018	543	360	115
West Virginia	11	5	6	
United States Army	1		1	
United States Navy	2		2	
Total	1,127	585	415	124

Most of the specimens submitted from distant States were shipped in some preserving medium, such as glycerin, formaldehyde, or other reagent.

TABLE II.—*Distribution of positive and negative specimens among different species of animals.*

Animal.	Total.	Positive.	Negative.	Not examined.
Dogs	922	514	306	102
Cats	103	34	54	15
Cattle	52	32	18	2
Horses	5	1	4	
Mules	4		4	
Swine	21	5	15	1
Sheep	2	1	1	
Skunks	5		5	
Squirrels	3		3	
Rabbits	2		2	
Opossum	1		1	
Rat	1		1	
Man	2	1		1
Unknown	4		1	3
Total	1,127	588	415	124

It is evident from Table II and from the natural proclivities of the animal to bite, that the dog (514 positive specimens out of a total of 588, is the greatest disseminator of rabies. It is also evident that the number of cattle (32), horses (1), and hogs (5) lost from the disease represents quite an economic loss. As most of the domestic animals represented, other than dogs and cats, do not often bite, it is probable that practically all the horses, cattle, sheep, and swine receive their infection as a result of being bitten by rabid dogs.

The seasonal prevalence of the disease in animals, as indicated by the results of this series, is shown in Table III.

TABLE III.—*Seasonal prevalence of rabies in animals.*

Year.	January.			February.			March.			April.			May.			June.			July.			August.			September.			October.			November.			December.			Total for year.
	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	Positive.	Negative.	Not examined.	
1909	....	....	....	2	....	....	....	....	....	....	....	....	....	1	....	1	1	1	1	3	....	1	....	....	1	1	....	3	....	....	2	1	....	....	1	....	20
1910	....	....	....	5	....	1	3	....	....	1	1	....	....	1	....	....	....	....	....	....	....	....	1	....	....	1	....	....	1	....	7	1	....	4	1	....	28
1911	1	1	....	1	....	....	2	....	....	2	1	....	5	3	....	3	4	....	1	1	....	3	1	1	4	1	1	5	2	....	6	3	....	4	2	....	58
1912	2	4	1	2	3	....	6	1	2	5	5	2	9	3	4	5	3	2	6	5	4	2	6	4	4	2	5	3	2	....	11	5	....	7	2	....	127
1913	5	1	2	6	2	....	9	1	1	7	5	....	8	2	....	7	4	1	6	8	....	4	2	2	2	4	1	4	3	1	6	3	1	7	3	....	118
1914	3	....	....	4	3	....	9	1	....	6	4	....	2	5	....	4	11	1	6	11	4	6	5	2	2	2	2	2	3	....	3	3	....	3	2	1	110
1915	8	5	....	5	9	....	12	5	....	6	5	3	4	4	3	6	6	2	4	7	2	1	2	....	6	7	1	2	2	1	5	....	1	10	4	2	140
1916	6	4	....	11	6	....	10	14	....	6	6	1	3	12	1	2	6	2	4	5	3	3	7	1	6	2	3	11	5	1	4	2	....	8	2	....	157
1917	13	9	1	10	4	1	12	9	....	11	11	....	9	8	2	5	5	1	5	7	3	8	8	7	9	1	1	4	3	1	7	4	....	5	1	....	184
1918	13	1	....	7	4	....	5	4	2	2	4	2	6	8	1	6	6	2	4	2	9	2	5	7	3	2	3	4	1	2	3	4	1	7	2	1	135
1919	14	1	1	8	5	....	7	....	1	6	6	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	50
Total	65	26	5	61	36	2	75	35	6	52	48	9	46	47	11	39	46	12	37	49	25	30	37	24	34	23	17	38	22	6	54	26	3	55	20	4	1,127

From Table III it will be seen that the greater number of positive brains were obtained in the colder months, which is directly opposed to the popular belief that rabies is more prevalent in hot months.

Table IV shows the different months of the year arranged in the order of the highest average number of positive cases.

TABLE IV.—*Summary of Table III showing occurrence of rabies by months.*

Month.	Positive.	Negative.	Not examined.	Monthly average, positive.	Percentage positive of those examined.
March	75	35	6	6.87	68.2
January	65	26	5	5.90	72.0
December	55	20	4	5.50	73.3
February	61	36	2	5.56	62.1
November	54	26	3	5.40	67.5
April	52	48	9	4.73	52.0
May	46	47	11	4.60	49.5
June	39	46	12	3.90	46.4
October	38	22	6	3.80	63.3
July	37	49	25	3.70	43.0
September	36	23	17	3.60	61.0
August	30	37	24	3.00	44.8
Total	588	415	124		

A factor which may explain the apparent lower prevalence in summer is the popular belief that dogs become rabid only in warm weather. As a result of this belief, if any dog appears sick or abnormal in warm weather the animal is promptly killed and the head sent in for examination. Consequently, in warm weather some dogs that are not rabid are killed and submitted for examination. On the other hand, in cold weather the animal is not regarded with as great suspicion, and animals not rabid are kept until they recover, while those suffering from rabies die of the disease and the brains are then submitted for examination. Moreover, it will be noted that many specimens were not examined during the warm months; but it is reasonable to suppose that the number of positive brains undergoing decomposition while en route would be balanced by the loss of a proportionate number of negative specimens.

The foregoing tables show that 124 brains received were not examined. The reasons for this are as follows: In 18 cases the brain was destroyed in killing the animal, usually the result of shooting it at close range with a shotgun. In 7 cases the brain was practically destroyed in killing, and partly decomposed. In 98 cases the brain was decomposed to such an extent that examination was impossible. In 1 case the animal was brought to the laboratory alive, observed for two weeks with negative results, and then returned to the owner.

It will be noted that in most instances the reason for nonexamination was decomposition of the brain, and that cases not examined were more numerous in hot weather; hence, the necessity of icing the specimens which are shipped during hot weather is apparent.

It should also be stated that many specimens were in bad condition when received, but one or more tests were made on them and the results of these examinations are embodied in the figures of this report. In general, decomposition or softening of the brain tissues prevents the making of sections; it may cause premature death of inoculated animals and interferes very much with the making of satisfactory smears. On the other hand, it is often possible to obtain smear preparations from the hippocampus major of what seems to be a hopelessly decomposed brain. The hippocampus seems to be one of the last parts of the brain to break down.

Results obtained from the different types of tests.

The foregoing tables show that 124 specimens were not submitted to examination for reasons already stated. Of the 1,003 specimens examined, 153 were submitted to all three tests. The results, as recorded, are shown below in tabular form.

TABLE V.—*Results obtained from the specimens submitted to three tests.*

Number of specimens.	Smears.	Sections.	Animal inoculation.	Remarks.
5	+	+	+	
134	−	−	−	
2	−	+	+	Missed by one microscopic test.
8	−	−	+	Missed by both microscopic tests.
3	+	−	−	Inconsistent finding.
1	−	+	−	Do.
153 (total).				

From Table V it is seen that in 139 specimens the findings by all three tests were identical. In 2 specimens the presence of Negri bodies in smears was missed, but detected by the section method and confirmed by animal inoculation. In 8 specimens the presence of Negri bodies was missed by both microscopic methods, but demonstrated by the animal inoculation test. In 3 cases Negri bodies are recorded as present in the smears, though not found on section or inoculation; and in 1 case they are recorded as present in sections, though not demonstrated by smears or inoculation tests. These last four findings are apparently inconsistent, since the animal inoculation test is probably the most reliable of the three. This will be discussed later.

Two hundred and fifty-seven specimens were submitted to two tests with the results shown in Table VI.

TABLE VI.—*Results obtained from the specimens submitted to two tests.*

Number of specimens.	Smears.	Sections.	Animal inoculations.	Remarks.
10	+	+		
5	−	+		Missed by one microscopic test.
1		+	+	
37	+		+	
1		−	+	Missed by both microscopic tests.
20	−		+	
175	−		−	
6	−	−		
1		−	−	
1	+		−	Inconsistent.
257 (total).				

In Table VI there is one inconsistent finding. In 5 cases the smears failed to show Negri bodies, but sections of the same specimens revealed them. In 21 cases the presence of Negri bodies was not detected by the microscope, but animal inoculation proved positive.

The remaining 593 specimens were submitted to but one test. The results are set forth in Table VII.

TABLE VII.—*Results obtained from the specimens submitted to one test.*

Number of specimens.	Smears.	Sections.	Animal inoculation.
488	+		
5			+
85	−		
12			−
3		−	
593 (total).			

In 488 specimens the presence of Negri bodies was shown in smears and the examination terminated at this point. In 17 cases no attempt was made to make microscopic tests, chiefly on account of the specimens being badly disorganized brains resulting from the killing of the animals by violence. Of these 17 cases, 5 gave positive results by animal inoculation. Three specimens were examined in sections only, on account of the material having been placed in formaldehyde or other fixing reagent before being examined. These all gave negative results. In 85 cases smears were made and examined with negative results, the examination being terminated at this point. Most of these specimens were in varying degrees of decomposition. In some of these cases animal inoculation was attempted, but the concomitant contamination resulted in the death of the the animals within a few days and before rabies could have developed.

Discussion of results.

Considering the figures given in Tables V and VI we find that of 389 specimens which were submitted to the animal-inoculation test in addition to the microscopic tests, 29 (7.5 per cent) were found positive by inoculation test but negative by the microscope. Also, that of 410 specimens, 36 were found negative on examination by the smear method but later found positive in sections or by inoculation test, making the error of the smear method 8.8 per cent. These examinations were distributed among 11 different men who had charge of the work at various times, the number charged against each man being in proportion to the length of time he was assigned to this work.

The tables also show that in 5 cases (1.3 per cent) a positive finding was reported on microscopic examination which failed of confirmation by animal inoculation, making the total error of the microscopic tests 8.7 per cent. The cases which were reported as positive and which failed to show rabies in the inoculated animals can be attributed in part to an effort to avoid reporting a specimen as "doubtful" or "suspicious." In some of these cases the records show that there was doubt, but that the examiner deemed the microscopic evidence to indicate the presence of Negri bodies. The five specimens thus reported were distributed among four different men and each error was made in the early part of each man's service, showing the conservative tendency of a new man to consider doubtful cases as positive. To illustrate more fully, a copy of the record in each case is quoted.

"No. 127. March 26, 1912. Dog's head from Dr. ——, Staunton, Va. Negri bodies in smears. No Negri bodies in sections. Two guinea pigs innoculated April 8, 1912. Animals all right June 10, 1912. (No. 126 was received at the same time from the same person and this head was too decomposed to permit examination. No mention of the condition of No. 127 is made.)"

"No. 128. April 1, 1912. Dog's head from Dr. ——, Staunton, Va. (Same person that sent No. 126 and 127.) Negri bodies in smears. No Negri bodies in sections. Two guinea pigs inoculated April 8, 1912, one animal died April 9, 1912. No symptoms. Other animal all right on June 27, 1912."

"No. 260. March 31, 1913. Dog's head from Culpeper, Va. Smears negative. A few Negri bodies found in sections. Two guinea pigs inoculated April 2. Both pigs died April 3. Two more pigs inoculated April 3. One pig died September 16, 1913. No Negri bodies found. No symptoms."

"No. 332. November 6, 1913. Dog's head from ——, Richmond Va. A few suspicious bodies present in smears, sections negative. November 7, 1913, two guinea pigs inoculated. One pig died November 25, 1913."

"No. 537. June 1, 1915. Dog's head from ——, Shanghai, Va. Suspicious bodies found in smears. Reported positive. Two guinea pigs inoculated June 3, 1915. Both pigs well and discharged from observation on October, 30, 1915."

Though the animals inoculated from specimens 127 and 128 were observed only for about two and one-half months, they are called negative in this paper since the average incubation period in the 29 cases giving positive result in animal inoculation and negative result on microscopic test was 17.1 days; while in the 44 microscopically positive specimens which were submitted to the animal-inoculation test as a confirmatory procedure the average incubation period was 15.9 days.

The percentage of error as given above has been based upon the assumption that the animal inoculation test gives 100 per cent of positive results. ("Percentage of error" is used with reference solely to the relative accuracy of the three methods and not in the strict mathematical sense.) This, probably, is not always true. To illustrate, let us consider specimen 902, the brain of a cat, which was negative on microscopic test on August 23, 1917. On August 29 two guinea pigs were inoculated from this specimen and on September 22 one pig died. Examination of the pig's brain showed the presence of Negri bodies. The other pig remained well and observation was discontinued on May 18, 1918. Had the first pig died within 5 days of inoculation from extraneous causes the original specimen would have been considered negative. However, such occurrences are negligible and are probably due to error of technique in inoculating one of the animals.

It might be argued that the same proportion of erroneous diagnoses had been made in the 488 specimens reported positive and the 88 reported negative, which were submitted to microscopic test only. While this may be true for the 88 negative cases, many of which were not submitted to animal tests on account of the extremely poor condition of the specimen, it is hardly applicable to the 488 positive specimens. These constitute the easily diagnosed and undoubted cases showing Negri bodies, and if doubt existed they would have been submitted to the animal inoculation test and consequently would have been tabulated as specimens submitted to two or three tests.

The time required for the performance of the different tests is approximately as follows:

Smear test: One hour (frequently less) after arrival of specimen.

Section test: Three to four days (a hurried test can be done in a day but will usually be unsatisfactory).

Animal inoculation test: Ten days to six months. After 30 days the probability of a positive result is small.

In conclusion, therefore, it may be stated that, based on 1,003 specimens examined, the correct diagnosis may be obtained on the day of receipt of specimen in 90 per cent of the cases; within five days in about 92 per cent; and at the end of one month practically all will be determined. Hence a negative report on microscopic examination with subsequent inoculation of animals practically becomes a confirmed negative at the end of one month, though it is the rule at present to observe animals for six months.

Summary.

1. From February 1, 1909, to April 30, 1919, specimens from 1,127 animals suspected of rabies were submitted to the Hygienic Laboratory for diagnosis.
2. One hundred and twenty-four of the specimens received were in such a state of decomposition, or disorganization from violence, that no examination could be made.
3. Of the 1,003 specimens examined, 588 showed evidence of rabies, and 415 gave negative results.
4. Nearly 82 per cent of specimens submitted and 87.4 per cent of those giving positive results were from dogs.
5. The colder months of the year have furnished the greatest number of specimens and the greatest percentage of positive findings.
6. Of 389 specimens which were submitted to both microscopic and animal inoculation tests, the microscopic finding was confirmed by the animal test in 91.3 per cent of the cases. The 8.7 per cent error was divided as follows: 1.3 per cent were found positive on microscopic test and negative on inoculation, and 7.4 per cent negative on microscopic test and positive on inoculation.
7. Examination by the section method gives slightly more accurate results than by the smear method.
8. Of the 406 specimens inoculated into animals there were 79 positive results, the average incubation period being 16.2 days.

VENEREAL DISEASE CONTROL.

PUBLIC HEALTH SERVICE SEEKS COOPERATION OF DENTISTS.

In connection with the campaign for the control of venereal diseases by the various State boards of health acting in cooperation with the Public Health Service, a circular letter, a bulletin, and a pledge card are being sent to each dentist in the United States. He is asked to sign and return the pledge card on which is indicated his willingness to cooperate in this important public health work. The circular letter follows: